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Increasing The Piroxicam Solubility by Complex Formation with PEG 6000

Suhemi B Sembiring, Tedjo Yuwono, Iis Wahyuningsih
Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta

Abstract

Piroxicam is a potent analgesic-antipyretic agent, but it has a poor solubility in water. This properties usually could make problems in the absorption rate. This substance might be included in class II of Biopharceutic Classssification Systems. It means that the dissolution rate has a good correlation with the absorption rate. So the effort of increasing the solubility of piroxicam is indeed needed. The aim of this study was to increase the solubility of piroxicam by complex formation with PEG 6000. The test were carried out by dissolving of piroxicam in the solutions containing 0,000 M; 0,00167 M; 0,00208 M; 0,0025 M; 0,00292 M and 0,00333 M of PEG 6000 in water at 32 °C, 37 °C and 42 °C. The effect of complex formation to the piroxicam solubility determined by spectrophotometer and the complex formation was detected by infrared spectroscopy. Then the result of solubility identified the complex stability constants and thermodynamic parameter. It was evident that the solubility of piroxicam in water increased significantly by adding the PEG 6000 because of the complex formation. The stability constants at 32 °C, 37 °C and 42 °C were: 199,19 M; 145,01 M and -49,07 M respectively, while the ΔH was $-1,595 \times 10$ cal mol, the ΔF were -3210,43 cal mol and -3064,44 cal mol and the ΔS was 10,52 cal mol and 9,88 cal moldeg at these temperatures. The complex of piroxicam-PEG 6000 was exothermic process, since the elevated temperature its solubility decreased. The data of infrared spectroscopy indicated that the complex formation of piroxicam-PEG 6000 was formed.

Key words : Piroxicam, PEG 6000, Complex, Solubility

Introduction

The bioavailability of a drug is associated with its therapeutic effect, clinical and toxicity activity. In orally therapeutic application usually, the solid drug will undergo, disintegration phase from its dosage form disaggregated, drug released, dissolved, and then to be absorption through the membrane to systemic system. For drugs with poor solubility, the dissolution rate become the determiner of its bioavailability (Shargel, *et.all*, 2005).

Piroxicam is a potent analgesic-antipyretic, anti rheumatic and anti inflammatory agent (Siswandono and Soekardjo, 2000) but it has poor solubility in water (Anonim, 1995). The poor solubility of drug tends to have a slow and imperfect absorption (Ansel, 2005), so the effort to increase the solubility is needed.

Some studies have been carried out in order to increase the drug solubility and bioavailability. Some methods can be used to increase drug solubility, such as salting forming, polymorphic or by adding some adjuvants as a

complex formation substance, surfactant and co solvent. (Yalkowsky, 1981).

One of choose method to increase the solubility is complex formation. Polyvinylpyrrolidone (PVP), α -cyclodextrin, polyethylene glycol 4000 (PEG 4000) and polyethylene glycol 6000 (PEG 6000) proved can increase the dissolution rate by complex formation (Anonim, 1986). Polyethylene glycol 6000 (PEG 6000) is a polar stable substance which is used as the adjuvant in the complex formation method. From its chemical structure that piroxicam and PEG 6000, can be predicted to increase piroxicam solubility by complex formation.

Considering that piroxicam has a poor biopharmaceutical properties this problem will be overcome by complex formation with PEG 6000.

Materials and Method

Materials

The main material in this research is piroxicam (pharmaceutical degree) (Calao), Polyethylene glycol 6000 (PEG 6000) pharmaceutical degree (NOF CORPORATION (got from PT. Otto Pharmaceutical Industries). Distillated water CO₂ free (UAD Analytical chemistry laboratories), NaOH analytical degree (E Merck), methanol analytical degree (E Merck).

Procedure

A. Calibration curve

Made a series concentration solution with and without PEG 6000 0,25 mg%; 0,5 mg%; 0,75 mg%; 1,0 mg%; 1,25 mg%; 1,5 mg% and 1,75 mg%.

B. Piroxicam solubility test in some PEG solution in varied concentration was assged at different temperature.

C. Orientation on saturation point of piroxicam solution.

The excessive amount of piroxicam (\pm 10mg) was added to the 35 ml distilled water CO₂ free in the solubility test equipment, then put some PEG 6000 solution assay, and stir them using the magnetic stirrer. The water circulation of thermostat waterbath used to arrange the temperature. This trial was done at 32 °C, 37 °C and 42 °C. Some samples from the solution was assayed at 15, 30, 45, 60, 75, 90, 105, 120 minute, until it reached the saturatied solutions. The samples were taken by decreasing the pressure using *disposable polhyethylene syringe* 40 ml through *milipore filter 0,45*, then measuring the absorbance using spectrophotometer UV. The trial was done until the saturation point was reached and on the needed time as the baseline in other trial.

D. Piroxicam solubility test on several PEG 6000 solution assays.

This solubility test was conducted same as the orientation trial but the sample were carried out after the saturation point time was reached. The sampling was conducted five times with interval time a half an hour, and three times replication.

The obtained absorbance was used to measure the dissolved piroxicam assay using the calibration curve linear regression.

E. Determining the interaction between piroxicam with PEG

The interaction between piroxicam and PEG 6000 was analyzed by comparing infrared spectrum the physical combination using rate piroxicam and PEG 6000 co-precipitant (1:1). To prepare the pyroxicam-PEG 6000 complex was carried out by dissolving both materials in methanol and destilled water CO₂ free, then evaporated the solvent and the presipitant will be formed. After that, the presipitant was centrifuged and dried in exicator namely co-presipitate. Both material (the complex and the physic combination) were compressed to be pelleds, and determined by infrared spectrofotometer.

Analysis of the Research Data

A. Piroxicam solubility

Piroxicam solubility data was analyzed using *Kolmogorof-smirnof* statistical test to know whether the data were normally distributed or not, and being continued with *Levene test* to know whether the data were homogen or not.

Result and Discusion

Piroxicam calibration curve

The piroxicam calibration curve in the variation concentration of PEG 6000 solution were made at λ max 353,4 nm, then linier regressions from the calibration curve was made as shown in table I.

Table I. Calibration curve equation of piroxicam in 6 different concentration of PEG 6000

PEG 6000 concentration (M)	Calibration cure equation	r analyzed	r table
0,00000	$Y = 0,4378 X + 0,0023$	0,9996	0,811
0,00167	$Y = 0,4368 X - 0,0014$	0,9992	0,811
0,00208	$Y = 0,4497 X - 0,0237$	0,9996	0,811
0,00250	$Y = 0,4621 X + 0,0001$	0,9996	0,811
0,00292	$Y = 0,4410 X - 0,0153$	0,9996	0,811
0,00333	$Y = 0,4589 X - 0,0073$	0,9994	0,811

The analysis was continued to know the significance of the data using *Anova* test and *t* test if the data were distributed normally and homogen, but the *Kruskal Wallis* test and *Mann-Whitney* test was conducted if the data were not distributed normally and homogen. Linear equation of concentration PEG 6000 and piroxicam solubility was analyzed by comparing the coefficient correlation value between *r* analyzed with *r* table with $p = 95\%$.

B. Determining the complex stability constant value and thermodynamic parameter

The obtained data from solubility test were used to determine the complex stability constant value and thermodynamic value.

C. The interaction between piroxicam and PEG 6000

Based on the result of the infrared spectrum between physical combining and PEG 6000, and co-precipitate of piroxicam and PEG 6000, then the complexes will be shown.

Based on those equation, correlation coefficients were higher than *r* table ($p=0,05$), so that the calibration curve equation can be used in piroxicam assay.

From the *Anova* test of all the calibration the result were not significant different. So that any of one of these equation can be used, nevertheless all of the equation were used to minimize the mistake on calculating the piroxicam solubility.

Piroxicam solubility test on several PEG 6000 solution assay at 32 °C, 37 °C and 42 °C

The trial orientation of piroxicam saturation showed that the saturation time was optained within five hours, so the sampling was carried out after five hours.

The results of piroxicam solubility test in the PEG 6000 solution (M) at 32 °C were shown on table II. The association between piroxicam solubility (M) and the concentration of PEG 6000 (M) were increase. The higher PEG 6000 concentration (x) the higher solubility of

pyroxicam (y) with the linear regression equation $Y = 0,030524X + 0,000158$ with correlation coefficient (r) = 0,8237. The result of *Kolmogorof-smirnof* test obtained that the data were not distributed normally. The result of *Kruscall-Wallis* test showed that the piroxicam solubility in varried PEG 6000 concentration was significantly different. The result of *Mann Whitney* test showed that by adding of PEG 6000 at 32°C would increase the piroxicam solubility significantly but at PEG 6000 0,00000 M with 0,00167 M; 0,00208 M with 0,00250 M and 0,00292 M with 0,00333 M. These happened because the interval of the PEG 6000 assays was small.

Table II. The result of piroxicam solubility test in the PEG 6000 solution (M) at 32 °C

PEG 6000 (M) Concentration	The solubility of pyroxicam (M)
0,00000	$1,78 \times 10^{-4} \pm 4,43 \times 10^{-5}$
0,00167	$1,84 \times 10^{-4} \pm 4,24 \times 10^{-5}$
0,00208	$2,09 \times 10^{-4} \pm 4,45 \times 10^{-5}$
0,00250	$2,07 \times 10^{-4} \pm 4,43 \times 10^{-5}$
0,00292	$2,78 \times 10^{-4} \pm 4,08 \times 10^{-5}$
0,00333	$2,73 \times 10^{-4} \pm 4,53 \times 10^{-5}$

The results of piroxicam solubility in the PEG 6000 solutions at 37 °C on (M) were shown on table III. The correlation of piroxicam solubility and the concentrations of PEG 6000 was linear with equation $Y = 0,020663X + 0,000146$ with coefficient correlation (r) = 0,8520.

The result of *Kolmogorof-smirnof* test was obtained that the data were normally distributed, but in *Levene* test showed that the data were not homogen. The result of *Kruskal-Wallis* test showed that the piroxicam solubility in several PEG 6000 assays was significantly different. The result of *Mann Whitney* test showed that by adding of PEG 6000 at 37°C would increase the piroxicam solubility significantly but not at PEG 6000 0,00000 M with 0,00167 M; 0,00208 M with 0,00250 M and 0,00292 M with 0,00333 M.

Table III. The results of piroxicam solubility test in the PEG 6000 solution (M) at 37 °C

PEG 6000 (M) Concentration	The solubility of pyroxicam (M)
0,00000	$1,56 \times 10^{-4} \pm 3,02 \times 10^{-5}$
0,00167	$1,57 \times 10^{-4} \pm 2,67 \times 10^{-5}$
0,00208	$1,79 \times 10^{-4} \pm 2,94 \times 10^{-5}$
0,00250	$1,85 \times 10^{-4} \pm 2,82 \times 10^{-5}$
0,00292	$2,16 \times 10^{-4} \pm 2,89 \times 10^{-5}$
0,00333	$2,22 \times 10^{-4} \pm 2,81 \times 10^{-5}$

The results of piroxicam solubility in the solution of PEG 6000 at 42 °C shown on table IV. The correlation of piroxicam solubility and the concentrations of PEG 6000 was linear with equation $Y = -0,00917X + 0,000184$ with coefficient correlation (r) = -0,5305.

The result of *Kolmogorof-smirnof* test was obtained that the data were normally distributed, but in *Levene* test showed that the data were not homogen. The result of *Kruskal-Wallis* test showed that the piroxicam solubility in several PEG 6000 assays was significantly different. The result of *Mann Whitney* test showed that by adding of PEG 6000 at 37°C would increase the piroxicam solubility significantly but at PEG 6000 0,00000 M with 0,00167 M with 0,00250 M.

Table IV. The results of piroxicam solubility test in the PEG 6000 solution at 42 °C

PEG 6000 (M) Concentration	The solubility of pyroxicam (M)
0,00000 ⁻⁴	$2,01 \times 10 \pm 1,82 \times 10^{-5*}$
0,00167 ⁻⁴	$1,46 \times 10 \pm 2,09 \times 10^{-5}$
0,00208 ⁻⁴	$1,59 \times 10 \pm 2,16 \times 10^{-5}$
0,00250 ⁻⁴	$1,47 \times 10 \pm 1,74 \times 10^{-5}$
0,00292 ⁻⁴	$1,64 \times 10 \pm 2,41 \times 10^{-5}$
0,00333 ⁻⁴	$1,75 \times 10 \pm 2,15 \times 10^{-5}$

The trial result on three different temperatures showed that piroxicam solubility

was decreased by the increasing of temperature. It was because the dissolution process of complex piroxicam and PEG 6000 released the heat called exothermic. It showed that the temperature gave the impact on the piroxicam solubility in the solution of PEG 6000.

Determining the complex stability constant value and thermodynamic parameter at the variations temperatures

Determining the complex stability constant value (K) and thermodynamic parameter at several trial temperatures were conducted by making the regression linear between PEG 6000 concentration and piroxicam solubility, its slope and intercept were used to calculate K value. This K value was applied to calculate the thermodynamic parameter of complex forming process.

The interaction between piroxicam and PEG 6000 was analyzed using infrared spectroscopy of piroxicam (figure 1), PEG 6000 (figure 2), physical combination of piroxicam and PEG 6000 (figure 3), and co-precipitate of piroxicam and PEG 6000 (figure 4)

The interpretation of infrared spectra was emphasized on the bands peak shift, pattern, and spectra intensity of characterized wavenumber area of piroxicam, PEG 6000, physical combination of piroxicam and PEG 6000 and co-precipitate of piroxicam and PEG 6000 spectra. The interpretation was observed their peaks surrounding the area of 3650 – 3200 cm^{-1} (O-H stretching), 3500 – 3100 cm^{-1} (N-H stretching), 3000 – 2850 cm^{-1} (C-H stretching), 1800 – 1600 cm^{-1} (C=O stretching), and 1350 – 1150 cm^{-1} (S=O stretching) (Sastrohamidjojo

Tabel V. Complex stability constant value and thermodynamic parameter

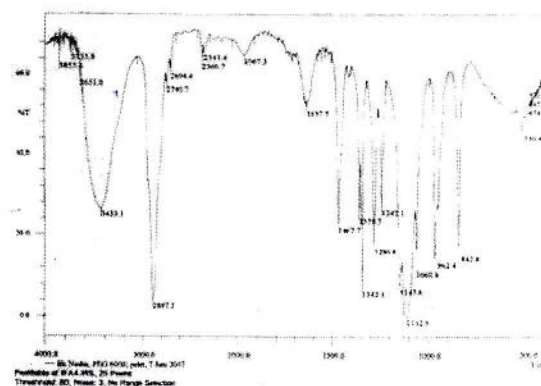
Temperature (°C)	Complex stability constant (K) (M^{-1})	Thermodynamic Parameter		
		ΔH (kal mol^{-1})	ΔF (kal mol^{-1})	ΔS (kal $\text{mol}^{-1} \text{der}^{-1}$)
32 °C	200	-12121	-3210	-29,2
37 °C	145	-12121	-3064	-29,2

When determining the complex stability constant value, the decreasing of complex stability constant value (K) by the increasing of temperature showed that the increasing of temperature decreased the formed complex or destabilized them.

When determining the thermodynamic parameters, were obtained the negative ΔH value, indicated that complex process was exothermic or released the heat, because the increasing of piroxicam temperature decreased the piroxicam solubility. The negative of ΔH , ΔF and ΔS values indicated that the complex formation process was spontaneous at low temperature (Martin, *et.all*, 1993).

The evaluation of Interaction between Piroxicam and PEG 6000 with Infrared spectroscopy

and Sardjoko, 1982). The peak shift, the important wavenumber, and the characteristic were shown in figure 5 and 6.



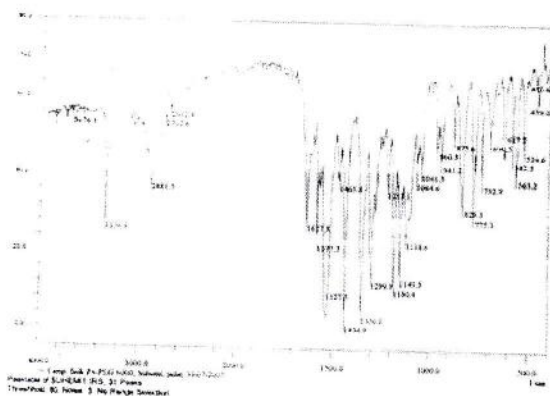


Figure 2. PEG 6000 infrared spectrum

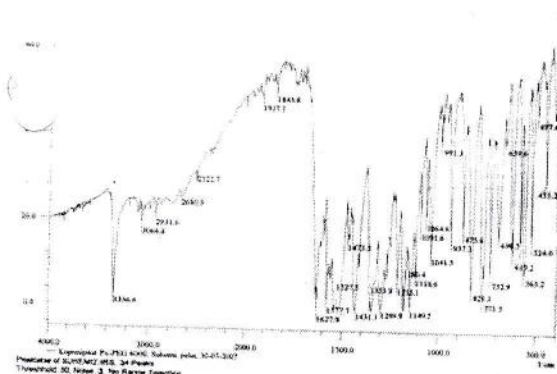


Figure 3. Physical combination of piroxicam and PEG 6000 infrared spectrum

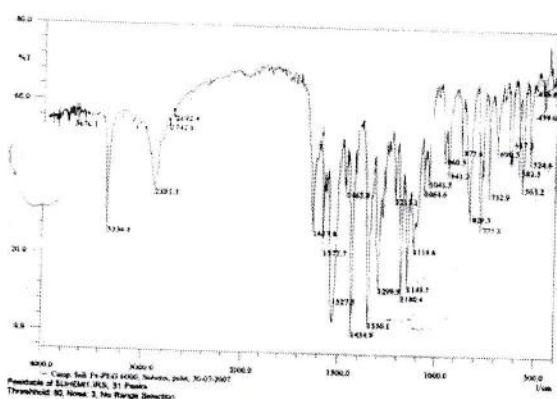


Figure 4. co-precipitate of piroxicam and PEG 6000 infrared spectrum

The spectra of single substances of piroxicam and PEG 6000, could be compared with the result of physical combination and co-precipitate of both substances. N-H amide stretching of physical combination gave a middle intensity and co-precipitate gave a high intensity emerged on $3336,6 \text{ cm}^{-1}$ wavelength. The appearance of secondary amide was shown at middle intensity of physical combination and at high intensity of co-precipitate emerged at $1577,7 \text{ cm}^{-1}$ wavelength. C=C stretching emerged at middle intensity of physical combination and at high intensity of co-precipitate at $1577,7 \text{ cm}^{-1}$. C-H aromatic stretching emerged at $3066,6 \text{ cm}^{-1}$ wavelength with co-precipitate spectrum at middle intensity but no physical combination spectra. S=O stretching emerged at $1350,1 \text{ cm}^{-1}$ wavelength with physical combination spectra at high intensity but no peak at the co-precipitate spectra. C-O stretching emerged at $1180,4 \text{ cm}^{-1}$ wavelength with middle intensity of physical combination spectra and high intensity of co-precipitate spectra. C=O stretching emerged $1627,8 \text{ cm}^{-1}$ wavelength with middle intensity of physical combination spectra and high intensity of co-precipitate spectra. O-H spectra of both physical combination and co-precipitate emerged at $3336,6 \text{ cm}^{-1}$ wavelength with high intensity.

From the picture, can be seen the spectra band shift of certain group. Those could be the evidence to analyze the complex of piroxicam and PEG 6000. Some physical combination and co-precipitate of piroxicam and PEG 6000 spectra were same. They showed that physical combination of piroxicam and PEG 6000 was also forming complex which possibly happened because it had a high pressure when was made a pellet with KBr.

Complex piroxicam with PEG-6000 occur through the electrostatic interaction as the result of their electron delocalization so that make possible the reaction happen. Piroxicam electron delocalization was shown on figure 5. The change of partial electron molecules of PEG 6000 happened because of the induction of O

atom which has electronegative characteristic, so that it can draw the electron surrounding atom C causing the partial positive charge on atom C and partial negative charge on O atom, figure 8. Those make the positive charge of piroxicam interacted with the negative partial charge of PEG 6000, so the opposite, so that the complex bound would occur as the result of the interaction.

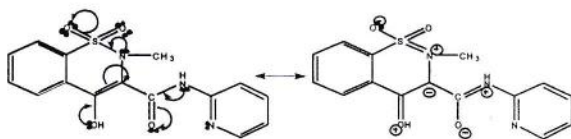


Figure 7. Piroxicam electron delocalization

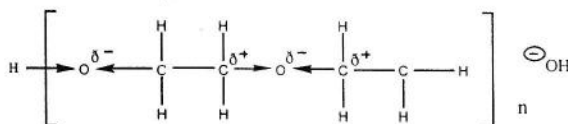


Figure 8. The effect of electro negativity of O atom to the partial charge of PEG 6000

Conclusion

1. Piroxicam can make complex with PEG 6000 marked by the increasing of piroxicam solubility.
2. Higher of PEG 6000 assay indicated more complexes were formed, so that the piroxicam solubility was increased.
3. There were increasing significantly of piroxicam solubility at 32 °C, 37 °C dan 42 °C by adding PEG 6000 assay. The complex forming of piroxicam and PEG were decreased with the increasing of temperature, so that the increasing of temperature would decreased the solubility of piroxicam.

4. Complex stability of piroxicam and PEG 6000 value (K) at 32 °C and 37 °C respectively 200 M⁻¹ dan 145 M⁻¹. thermodynamic parameters value in complex formation process were: DH = -12121 kal mol, value DF at 32 °C and 37 °C respectively -3210 kal mol⁻¹ and -3064 kal mol⁻¹, and both of them have the same DS value = -29,2 kal mol⁻¹ der⁻¹.

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